

Activated carbon does not prevent the toxicity of culture material containing fumonisin B₁ when fed to weanling piglets¹

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ABSTRACT: Fumonisin is a mycotoxin found primarily in corn and corn products that are produced by *Fusarium verticillioides*, *F. proliferatum*, and several other *Fusarium* species. The toxicity of fumonisin B₁ (FB) from culture material with and without activated carbon was evaluated using weanling piglets. Fifty-six weanling pigs were assigned to one of four treatment diets based on BW. The treatment diets were 1) control = corn-soybean basal diet with <2 ppm FB; 2) AC = control + activated carbon at 1% of the diet, as fed; 3) FB = control + culture material (formulated to contain 30 ppm FB, as-fed basis); and 4) AC + FB = control + activated carbon at 1% of the diet as fed + culture material (formulated to contain 30 ppm FB). A total of four replicates of four pigs per pen for the control and AC treatments and three piglets per pen for the FB and AC + FB treatments were used. Feed and water were offered ad libitum for the duration of the 42-d experiment. Compared with pigs fed the control or AC diets, pigs receiving the two FB-contaminated diets (FB or AC + FB) had lower G:F ($P < 0.01$), higher serum enzyme activities of γ -glutamyltransferase and glutamic oxaloacetic transaminase ($P < 0.05$), and higher concentra-

tions of cholesterol, free sphinganine, sphingosine-1-phosphate, and sphinganine 1-phosphate ($P < 0.05$). Although animals consuming FB diets showed no signs of respiratory distress, all pigs consuming either the FB or the AC + FB diets had marked pulmonary edema. Lesions were observed in the lungs, heart, and liver of pigs fed the FB or AC + FB diets, and treatment-associated changes also were seen in the pancreas, intestines, spleen, and lymph nodes. No lesions were observed in the brain. In liver, lung, heart, pancreas, spleen, intestines, and lymph nodes, the histopathological effects observed were more severe in the AC + FB group, suggesting that the AC treatment worsened the toxic effects of FB. Additionally, immunological measurements of macrophage function (CD14) were affected ($P < 0.05$) by the consumption of the FB diets. The consumption of FB diets containing 30 ppm fumonisin B₁ from cultured material significantly affected performance, biochemical measurements, and organ pathology in weanling pigs. The addition of activated carbon at the rate of 1% to the diet was not effective in protecting against the detrimental effects of fumonisin consumption.

Key Words: Fumonisin B₁, Mycotoxins, Sequestering Agents, Swine, Toxicity

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Introduction

Fumonisin is a group of mycotoxins that have been associated with toxicities in several species; these mycotoxins occur throughout the world, primarily in corn

and corn-based products. Fumonisin is produced primarily by *Fusarium verticillioides* and *F. proliferatum* (Shephard et al., 1996). Fumonisin has been shown to cause leukoencephalomalacia in horses (Marasas et al., 1988; Ross et al., 1993), liver and renal cancer in rodents (IARC, 2002), and porcine pulmonary edema (PPE) and hydrothorax in swine (Colvin and Harrison, 1992; Osweiler et al., 1992). The mechanism of action of

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fumonisin involves inhibition of the enzyme ceramide synthase, a key enzyme in the biosynthesis of sphingolipids (Wang et al., 1991). Fumonisin also may pose a potential risk to humans, as they have been associated with the high prevalence of human esophageal cancer in Southern Africa and China (Rheeder et al., 1992; Chu and Li, 1994).

The utilization of sequestering agents to adsorb aflatoxins and certain other mycotoxins has been identified as an effective and practical method for decreasing mycotoxin exposure in agricultural livestock (Ramos and Hernandez, 1997; Galvano et al., 2001); however, information on the ability of sequestering agents to bind mycotoxins other than aflatoxin is limited. In vitro data have shown that some sequestering agents effectively bind fumonisin (Galvano et al., 1997; Solfrizzo et al., 2001b), but their effectiveness in vivo has not been thoroughly examined. Several studies have determined that in vitro testing of these sequestering agents does not always correlate with their potential in vivo (Galvano et al., 1996; Solfrizzo et al., 2001a; Diaz et al., 2004).

This study was conducted to characterize the effects of long term feeding of fumonisin B₁ (FB) on the performance, immunocompetence, pathology, blood chemistry, and organ weights of weaned piglets and to evaluate whether activated carbon is effective in binding fumonisin and reduce its absorption and toxicity in animals.

Materials and Methods

Animals and Experimental Protocol

The institutional ethical committee on the care and use of laboratory animals of Università di Bologna reviewed and approved the experimental protocol. Fifty-six castrated male piglets (Landrace × Large White) with an initial BW of 6.9 kg (SE = 0.30) were blocked by BW and assigned randomly to 16 pens (1.80 m × 1.00 m) and to one of four dietary treatments. Pigs that were vaccinated for *Mycoplasma hyopneumoniae* (SUVAXYN MH-one, Fort Dodge Animal Health, Overland Park, KS) were purchased from a local supplier (Azienda Agricola Antigola, Loiano, Italy). Pigs were checked by a staff veterinarian upon arrival and periodically during the experimental period and were determined to be free of disease and in good health. All animals were fed a basal diet (control diet) for a 5-d adaptation period. The treatment diets were 1) a corn-soybean basal diet with <2 ppm FB (control); 2) control + activated carbon (SORBOPOR MV 125 Camel Envirotech S.r.l. Milan, Italy) at 1% (as-fed) of the diet (AC); 3) control + FB culture material (formulated to contain 30 ppm FB, as-fed basis); and 4) control + activated carbon at 1% of the diet (as-fed) + culture material (formulated to contain 30 ppm FB; AC + FB). A total of four replicates of four piglets per pen for the control and AC treatments and three piglets per pen for the FB and AC + FB treatments were used. The activated

carbon used in this experiment was obtained from various wood sources and had a surface area of 1,116 m²/g and an iodine number of 1,250 mg/g. This activated carbon previously had been tested in vitro with respect to its ability to bind FB (Galvano et al., 1997).

Experimental Diets

Fumonisin B₁ was produced by *F. proliferatum* cultured on whole corn as described by Smith et al. (2000). After thorough mixing, feeds were analyzed for fumonisin content as well as for other mycotoxins (aflatoxin, deoxynivalenol, zearalenone, and T-2 toxin). Diets were free of detectable levels of aflatoxin, deoxynivalenol, zearalenone, and T-2 toxin. For the fumonisin analysis, feed (25 g) was extracted with 50 mL of methanol:water (3:1). The mixture was then purified by solid-phase extraction on a strong anion-exchange cartridge (Isolute 500 mg). *o*-Phthaldialdehyde reagent was used for pre-column derivatization before chromatographic separation by isocratic reversed-phase HPLC with fluorescence detection according to the procedure of Sydenham et al. (1996). Feed and water were offered ad libitum for the duration of the 42-d experiment. Animals were observed daily for any adverse clinical signs. Diets were formulated to meet or exceed minimum nutrient requirements recommended by NRC (1998). Pigs were weighed, and feed disappearance was measured every 2 wk during the experimental period to determine ADG, ADFI, and G:F.

Clinical Chemistry

Once every 2 wk, blood samples (six per treatment; one pig per pen from two blocks, and two pigs per pen for the other two blocks) from randomly selected pigs fasted 16 h before sampling were collected via the anterior vena cava. Serum concentrations of total protein, albumin, globulin, glucose, cholesterol, triglycerides, bilirubin, urea, creatinine, Ca, P, Mg, and enzyme activities of glutamic oxaloacetic transaminase (GOT), and γ -glutamyltransferase (GGT) were measured using a DuPont automatic clinical analyzer (Dimension SMS-ES, DuPont Instruments, Wilmington, DE).

Sphingolipid Analysis

Blood samples from six animals per treatment (one pig per pen from two blocks, and two per pen for the other two blocks) as well as urine (from urinary bladder at necropsy) were collected on the last day of the experiment. Serum samples were analyzed for sphingoid bases and the sphingoid base 1-phosphates. The method for extraction of free sphingoid bases and their 1-phosphates from serum was a modification of the method of Sullards and Merrill (2001). Briefly, serum was combined with methanol:chloroform (2:1). The mixture was capped tightly, sonicated, and incubated at 48°C. After 16 h, the mixture was cooled, and 1 M methanolic KOH was added, sonicated, capped tightly, and incubated for

2 h at 37°C. After 2 h, the mixture was centrifuged, and the supernatant fraction was transferred to a clean glass tube and evaporated to dryness in a SpeedVac (ThermoSavant, Holbrook, NY). The residue was reconstituted in acetonitrile:water:formic acid (49.5:49.5:1) containing 5 mM ammonium formate and clarified by filter centrifugation.

The analysis of free sphinganine, free sphingosine, sphinganine 1-phosphate, and sphingosine 1-phosphate was done by liquid chromatography tandem mass spectrometry. Sphingoid bases and sphingoid base 1-phosphates were chromatographically separated on a Thermal Separations (Riviera Beach, FL) HPLC using an Intersil 5- μ m ODS-3 column (150 \times 3 mm; Metachem Technologies, Inc., Torrance, CA). The mobile phase was a mixture of 97% acetonitrile:2% water:1% formic acid (Solvent A) and 97% water:2% acetonitrile:1% formic acid (Solvent B), with a flow rate of 0.2 mL/min. The gradient started at 50% Solvent A, at 15 min it was 70% Solvent A, and at 20 min, it was 100% Solvent A, which was held until 25 min, at which time the column was reequilibrated with 50% Solvent A. The total run time was 40 min. The column effluent was directly coupled to a ThermoFinnigan LCQ Duo ion trap mass spectrometer (MS) (Woodstock, GA). The MS was operated in the electrospray ionization (ESI) positive ion mode with an inlet capillary temperature of 170°C and the sheath gas was N₂ (50 arb). For MS/MS, the collision energy was 30%, and the parent *m/z* for MS/MS and single ion monitoring mode were 300.2, 302.2, 380.5, and 382.5 for sphingosine, sphinganine, sphingosine 1-phosphate, and sphinganine 1-phosphate, respectively. For MS/MS, mass fragments were scanned from 195 to 400 *m/z* and compared to authentic standards. Quantification was accomplished using both internal and external standardization. The internal standard was based on the recoveries of the C20-sphinganine added during extraction. Sphinganine and sphingosine were purchased from Sigma Chemical (St. Louis, MO), and sphingosine-1 phosphate and sphinganine-1 phosphate were from Biomol Research Laboratories (Plymouth Meeting, PA). The C20 dihydro-sphingosine internal standard was a gift of A. Merrill, Jr., and D. Liotta (Georgia Institute of Biology and Emory University, Atlanta, GA).

Sphinganine and sphingosine of centrifuged urine samples were determined by reverse-phase HPLC with fluorescence detection after liquid-liquid extraction, silica gel column clean-up, and precolumn derivatization with *o*-phthaldialdehyde reagent as described by Solfrizzo et al. (1997, 2001a).

Pathology

At the end of the experimental period, six pigs per treatment (one pig per pen from two blocks and two per pen for the other two blocks) were killed according to approved electrocution and exsanguination procedures (FASS, 1999), and necropsies were performed. The

liver, kidney, and pancreas were weighed, and the organ weight relative to metabolic BW was calculated (g/kg of BW^{0.75}). Liver, spleen, kidney, heart, lymph nodes, lung, brain, pancreas, and intestinal samples were sectioned for pathological analysis. These samples were immediately fixed in 10% neutral buffered formalin, imbedded in paraffin, sectioned at a thickness of 5 μ m, stained with hematoxylin and eosin, and analyzed microscopically.

Flow Cell Cytometric Analysis

Heparinized blood from four animals, selected randomly in the control, FB, and AC + FB treatment groups (one from each pen) were collected via the anterior vena cava before euthanasia. The mononuclear fraction, including T-lymphocytes was isolated by density centrifugation using Histopaque 1077 (Sigma Chemical). Leucocytes (white blood cells) recovered from the interface (buffy coat) were washed twice with sterile PBS. Erythrocytes (red blood cells) were lysed using 0.15 M NH₄Cl (pH 7.2) for 10 min at room temperature. The purified mononuclear cells were then resuspended in RPMI-1640 (Sigma Chemical) medium supplemented with 10% fetal bovine serum.

For each sample 1,000,000 cells were recovered and incubated with either primary anti-CD4 (mouse anti-porcine CD4, Mab 74-12-4; VMRD, Pullman, WA); anti-CD8 (mouse anti-porcine CD8, MCA 1223; SEROTEC, Raleigh, NC), or anti-CD14 (mouse anti-porcine CD14, CAM36A; VMRD) for 30 min in a 0.5% PBS/BSA solution at 4°C. At the end of the incubation, the cells were washed with PBS solution and then reincubated, with secondary anti-mouse fluorescein isothiocyanate-conjugated (DAKO, Copenhagen, Denmark) for 30 min in 0.5% PBS/BSA at 4°C. Cells were then washed with PBS and analyzed by flow cytometry (Coulter Epics XL-MCL, Fullerton, CA).

Statistical Analyses

This experiment used a randomized complete block design, which included the fixed effects of dietary treatment and block. Pen served as the experimental unit for all performance data. Data that included only one sample per experimental unit were analyzed by ANOVA as a randomized complete block design using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Data that included multiple measurements per experimental unit were analyzed by repeated measures ANOVA as recommended by Littell et al. (1998), using the MIXED procedure of SAS. In both types of analysis, least squares means for treatments were separated using the LSMEANS statement and the PDIF option of SAS. Statistical significance was declared at *P* < 0.05.

Results

Performance, Growth, Feed Intake, and Organ Weights

There were no statistical differences in ADFI among treatment groups; however, G:F and ADG were de-

Table 1. Effects of fumonisin B₁ consumption (0 or 30 ppm) with or without the addition of activated carbon (1% of the diet, as-fed basis) on growth performance and organ weights of weanling pigs^a

Item	Treatment ^b				SEM	P-value
	Control	AC	FB	AC + FB		
Initial BW, kg	7.03	6.93	6.91	6.91	0.30	0.992
ADG, g	470.9 ^x	412.8 ^{xy}	326.6 ^{yz}	256.3 ^z	38.24	0.009
ADFI, g ^c	958.2	937.8	1,002.3	886.7	62.59	0.647
G:F	0.49 ^x	0.45 ^x	0.33 ^z	0.29 ^z	0.04	0.009
Organ weight ^d						
Liver	7.57	7.35	8.41	7.78	0.288	0.090
Kidney	1.57	1.43	1.46	1.34	0.072	0.182
Pancreas	0.74	0.41	0.68	0.68	0.125	0.282

^aEach value represents the mean of four pens, with three or four pigs per pen.

^bControl = corn-soybean basal diet with <2 ppm FB; AC = control + activated carbon at 1% of the diet, as-fed basis; FB = control + culture material (formulated to contain 30 ppm fumonisin B₁, as-fed basis); AC + FB = control + activated carbon at 1% of the diet, as-fed basis + culture material.

^cAs-fed basis.

^dOrgan weight relative to metabolic BW (g/kg of BW^{0.75}).

^{x,y,z}Within a row, means without a common superscript letter differ, $P < 0.05$.

creased ($P < 0.01$) by consumption of diets (FB and AC + FB) containing 30 ppm fumonisin (Table 1). In the FB group, the ADG was decreased relative to the control treatment group; however, the decrease was not significant compared with the AC group, nor was it significantly different compared with the AC + FB treatment group (Table 1). The addition of activated carbon, at 1% of the diet, did not significantly affect ADG, ADFI, or G:F, nor did it alleviate the effects associated with fumonisin consumption. Liver, kidney, and pancreas weights were not affected significantly by the consumption of the FB diets or by the consumption of the activated carbon (Table 1). Animals consuming the FB diets had no clinical signs of lung problems (i.e., difficulty in breathing).

Blood Chemistry

Serum concentrations of total protein, bilirubin, glucose, urea, albumin, creatinine, Ca, P, and Mg were not significantly affected by dietary addition of FB, AC, or AC + FB (Table 2). Serum concentrations of GGT, GOT, and cholesterol were increased ($P < 0.01$) by consumption of the FB diet, and there was no improvement in these measurements when activated carbon was added to the FB diet (Table 2).

Pathology

A summary of the microscopic and macroscopic pathology observations in all experimental groups evaluated are presented in Table 3. Pigs consuming FB diets with and without the addition of activated carbon showed distinct lesions in the lungs, heart, and liver. Animals consuming the AC + FB diet had the most severe lesions in these organs. Although animals consuming FB diets showed no clinical signs of respiratory problems (e.g., difficulty in breathing), all pigs consum-

ing either the FB or the AC + FB diets had marked pulmonary edema. Pigs consuming FB diets with or without the addition of activated carbon had visible discoloration (fibrosis) of the liver, vacuolation, and necrosis (including occasional single cell necrosis), although no differences ($P < 0.09$) were observed in liver weights.

Sphingolipid Alterations

Serum concentrations of free sphinganine and the sphingoid base 1-phosphates were increased significantly in animals consuming FB diets with or without the addition of activated carbon (Table 4). Sphinganine to sphingosine ratios (**Sa:So**) also were increased in serum of animals consuming the FB and AC+FB diets; however, the increase in free sphinganine and the Sa:So ratio was significantly less in the AC + FB group than in the FB group (Table 4). Urinary concentrations of sphinganine and the ratio of sphinganine to sphingosine were affected similarly by the dietary addition of fumonisin at 30 ppm (Table 5).

Immunological Measurements

Pigs consuming FB diets with or without the addition of activated carbon had decreases ($P < 0.01$) in CD14 antigen compared with pigs fed the control diet (Table 6). Additionally, a trend for a decrease in CD4 subpopulations was observed between the control and the FB group. Subpopulations of CD8 were drastically decreased in animals consuming the AC + FB diet; however, there was no significant decrease in the number of animals consuming FB alone. In animals receiving the AC + FB diet, the decrease in CD4 was more pronounced than in animals receiving FB alone (Table 6).

Table 2. Effects of fumonisin B₁ with or without the addition of activated carbon on serum chemistry of weanling pigs^a

Item	Treatments ^b				SEM	P-value
	Control	AC	FB	AC + FB		
GOT, IU/l ^c	69.5 ^x	56.9 ^x	304.6 ^y	396.1 ^y	43.8	<0.001
GGT, IU/l ^d	35.9 ^x	42.1 ^x	123.6 ^y	134.1 ^y	27.0	0.009
Bilirubin, µmol/L	8.1	9.1	13.7	18.1	4.2	0.161
Glucose, mmol/L	5.2	5.2	5.6	5.1	0.3	0.567
Cholesterol, mmol/L	2.0 ^x	2.3 ^x	3.2 ^y	3.4 ^y	0.2	<0.001
Protein, g/dL	5.7	5.4	6.0	5.8	0.2	0.077
Urea, mmol/L	3.2	3.1	3.0	3.2	0.3	0.893
Albumin, g/dL	3.1	2.9	2.7	2.8	0.1	0.112
Creatinine, mg/dL	1.0	0.9	1.2	1.2	0.1	0.076
Ca, mmol/L	2.7	2.6	2.9	2.9	0.1	0.178
P, mmol/L	2.5	2.6	1.9	2.1	0.3	0.217
Mg, mmol/L	0.9	0.8	0.9	1.0	0.1	0.371

^aEach value represents the mean of four pens, with three or four pigs per pen.

^bControl = corn-soybean basal diet with <2 ppm FB; AC = control + activated carbon at 1% of the diet, as-fed basis; FB = control + culture material (formulated to contain 30 ppm fumonisin B₁, as-fed basis); AC + FB = control + activated carbon at 1% of the diet, as-fed basis + culture material.

^cGlutamic oxaloacetic transaminase.

^dGamma glutamyl transferase.

^{x,y,z}Within a row, means without a common superscript letter differ, *P* < 0.05.

Discussion

Studies on the toxicity of FB in pigs have been done using either purified FB (Haschek et al., 1992; Prelusky et al., 1996; Rotter et al., 1996), cultured material containing FB (Casteel et al., 1993; Colvin et al., 1993; Fazekas et al., 1998; Gumprecht et al., 1998; Smith et al., 1999; Zomborszky et al., 2000; Zomborszky-Kovacs et al., 2002), or with naturally contaminated feeds (Haschek et al., 1992; Motelin et al., 1994). In the present study, consumption of diets containing 30 ppm of FB (from cultured material) decreased ADG and G:F and increased serum activities of the liver enzymes GOT and GGT and cholesterol in postweaned piglets fed for 42 d, but did not significantly affect weights of liver, kidneys, and pancreas. Motelin et al. (1994) reported similar effects on serum enzymes indicative of liver damage and elevated cholesterol when they fed weaned piglets diets with 175 and 101 ppm FB. Rotter et al. (1996) and Zomborszky-Kovacs et al. (2002) also found increased GOT, but at much lower fumonisin concentrations (10 ppm) than in the present study. Unlike the data of Zomborszky et al. (2002), but similar to that of Rotter et al. (1996), animals consuming the FB diets had significant decreased ADG and G:F. The addition of the activated carbon was not effective in preventing the decreased performance effects associated with fumonisin consumption. Hypercholesterolemia noted in our experiment and supported by several others experiments (Casteel et al., 1993; Colvin et al., 1993; Haschek et al., 1992; Rotter et al., 1996) tends to be an early clinical pathological sign in pigs consuming fumonisin-contaminated diets.

Zomborszky-Kovacs et al. (2002) saw a significant effect on lung weight and clear pulmonary edema when

piglets consumed a diet contaminated with 40 ppm FB, although the animals showed no clinical signs of lung problems (i.e., difficulty in breathing). Similarly, in our study, pulmonary edema and interstitial pneumonia were observed in all animals consuming fumonisin-contaminated diets without the presence of clinical signs. Unfortunately, due to problems during the sample collection, lung and heart weights were not accurately measured.

Pigs consuming FB diets with and without the addition of activated carbon for 42 d showed significant lesions in the lungs and liver similar to those observed by others (Casteel et al., 1993; Colvin et al., 1993; Fazekas et al., 1998; Gumprecht et al., 1998; Smith et al., 1999; Zomborszky et al., 2000; Zomborszky-Kovacs et al., 2002). Although FB has a low bioavailability, what is absorbed is widely distributed throughout the animal (Prelusky et al., 1996), and treatment-associated changes were detected in the pancreas, intestines, spleen, and lymph nodes. No lesions were observed in the brain. In liver, lung, heart, pancreas, spleen, intestines, and lymph nodes, the histopathological effects were more severe in the AC + FB group (Table 3), suggesting that the AC treatment worsened the toxic effects of FB. Villious fusion and atrophy in the intestines of FB-fed piglets are suggestive of viral disease; however, we have no evidence to support that conclusion, as the control and AC animals did not show any evidence of such lesions. The lesions in heart tissue are particularly noteworthy because it is believed that disruption of cardiovascular function plays an important role in the induction of PPE (Smith et al., 1999, 2000; Constable et al., 2000).

Increased serum and urinary concentration of free sphingoid bases, as demonstrated by other researchers

Table 3. Pathological observations in organs of pigs consuming fumonisin B₁ with or without the addition of activated carbon (1% of the diet, as-fed basis).

Organ	Treatments ^a			
	Control	AC	FB	AC + FB
Lungs	No lesions (normal structure)	No lesions	Focal interstitial pneumonia and diffuse edema	Same as FB with more severe lesions
Heart	No lesions	No lesions	Frequent loss of striation and myofiber fragmentation; microvacuolation; rare infiltration of small lymphocytes	Same as FB with more severe lesions
Liver	No lesions	No lesions	Macroscopically: irregular coloration of the liver surface and compact consistency. Microscopically: frequent disorganization of hepatic laminae and mild peribulbar fibrosis	Same as FB with more severe lesions
Pancreas (exocrine)	No lesions	No lesions	Frequent cytoplasmic micro and macrovacuolation, focal glandular hyperplasia	Same as FB with occasional interstitial infiltration of lymphocytes
Kidney	No lesions	No lesions	Moderate cloudy degeneration of renal tubules	No lesions
Intestine (duodenum, jejunum, ileum)	Mild infiltration of lymphocytes, plasma cells and monocytes; presence of submucosal nodular lymphoid aggregates	Same as control with moderate infiltration of lymphocytes, plasma cells and monocytes	Severe infiltration of lymphocytes and monocytes; villous fusion and atrophy; moderate infiltration of eosinophils and presence of submucosal nodular lymphoid aggregates	Same as FB with more severe lesions; mild lymphoid aggregation
Spleen	No lesions	Pigment laden macrophages	Mild lymphoid hypoplasia	Moderate lymphoid hypoplasia and pigment laden macrophages
Lymph nodes (meseraic)	Macroscopically: no lesions. Histologically: lymphoid hyperplasia	Occasional presence of pigment laden macrophages	Mild lymphoid hypoplasia	Moderate lymphoid hypoplasia and pigment laden macrophages
Brain	No lesions	No lesions	No lesions	No lesions

^aControl = corn-soybean basal diet with <2 ppm FB; AC = control + activated carbon at 1% of the diet, as-fed basis; FB = control + culture material (formulated to contain 30 ppm fumonisin B₁, as-fed basis); AC + FB = control + activated carbon at 1% of the diet, as-fed basis + culture material.

(Haschek et al., 2001; Solfrizzo et al., 2001b) is a specific biomarker of fumonisin exposure in all species in which it has been tested (WHO, 2000). Riley et al. (1993) reported that changes in free sphingoid bases were a sensitive biomarker for fumonisin exposure in swine and that these changes are detectable before the development of other biochemical and pathological measurements. In the AC + FB animals, the elevation in free sphinganine was significantly less than in the animals consuming FB alone; however, the total of free sphinganine and sphinganine 1-phosphate was similar for both groups (Table 4). Thus, the degree of fumonisin inhibition of ceramide synthase as indicated by the total

(molar) accumulated sphinganine was similar for both the FB alone and the AC + FB groups. To the best of our knowledge, this is the first report of fumonisin-induced elevation in sphingoid base 1-phosphates in animals fed diets containing fumonisin. This is important for two reasons. First, sphinganine 1-phosphate, like free sphinganine, is extremely low in serum; however, the increase is greater than free sphinganine. Thus, the elevation in sphinganine 1-phosphate may prove to be a more reliable marker for FB exposure in animals. Second, because sphingoid base 1-phosphates are known specific ligands for G protein-coupled receptors (Spiegel and Milstien, 2002) found in cardiovascu-

Table 4. Effects of fumonisin B₁ consumption with or without the addition of activated carbon (1% of the diet, as-fed basis) on serum-free sphingoid bases and sphingoid base 1-phosphates of weanling pigs

Item	Treatments ^a				SEM	P-value
	Control	AC	FB	AC + FB		
Free sphingosine (So), nM ^b	655	931	841	753	161	0.662
Free sphinganine (Sa), nM	59 ^b	58 ^b	945 ^d	590 ^c	118	<0.001
Free Sa:free So ratio	0.1 ^b	0.1 ^b	1.2 ^d	0.8 ^c	0.1	<0.001
Sphingosine 1-phosphate, nM	3,795 ^b	4,472 ^b	8,525 ^c	8,245 ^c	1,396	0.001
Sphinganine 1-phosphate, nM	70 ^b	91 ^b	1,712 ^c	2,086 ^c	372	0.048
Total sphingosine ^c	4,450	5,404	9,368	9,002	1,436	0.054
Total sphinganine	129 ^b	149 ^b	2,656 ^c	2,676 ^c	389	<0.001

^aControl = corn-soybean basal diet with <2 ppm FB; AC = control + activated carbon at 1% of the diet, as-fed basis; FB = control + culture material (formulated to contain 30 ppm fumonisin B₁, as-fed basis); AC + FB = control + activated carbon at 1% of the diet, as-fed basis + culture material.

^bFree = sphingosine and sphinganine extracted from serum with their primary amino group unmodified.

^cTotal = the sum of sphinganine or sphingosine as the free sphingoid base and their respective sphingoid base 1-phosphate expressed as the nanomolar concentration.

^{x,y,z}Within a row, means without a common superscript letter differ, $P < 0.05$.

lar tissue, and because sphingosine 1-phosphate also is a second messenger in pathways regulating Ca homeostasis (Spiegel and Milstien, 2002), the marked elevation in both sphingosine 1-phosphate and sphinganine 1-phosphate could contribute to the cardiovascular effects that are hypothesized to be the cause of fumonisin-induced PPE (Smith et al., 1999, 2000; Constable et al., 2000).

Urinary free sphinganine and sphingosine concentrations were highly variable in our experiment, which could be explained by the fact that only mild kidney lesions were observed in animals consuming the FB diet. Changes in free sphingoid bases in urine are due to the sloughing off of cells from the collecting ducts in the kidney (Riley et al., 1994; Hard et al., 2001). Thus, increased apoptosis or necrosis in the kidney, which was not observed in our experimental animals, is necessary to detect marked elevation of urinary sphinganine. The elevation in urinary and serum sphingoid base concentrations confirmed that activated carbon did not prevent uptake of fumonisin from the gut and had no protective effect against fumonisin-induced inhibition of ceramide synthase. Similarly, Solfrizzo et al. (2001a) reported no change in Sa:So in rats consuming FB with or without the inclusion of activated carbon.

Cell-mediated immune function effects with a decrease in macrophage function in swine exposed to fumonisin have been described previously. Muller et al. (1999) described a decrease in the number of phagocytic monocytes and in the numbers of polymorphonuclear neutrophils in weanling pigs. Theumer et al. (2002) examined rats intoxicated for 3 mo with FB, in which they noted a strong suppression of macrophage function with elevated concentrations of IL-4 and a decrease in IL-10, which the authors concluded was related to the immunosuppressive effects that fumonisin can have on macrophage immunologic mechanisms. Animals consuming the FB and the AC + FB diets had decreased concentrations of CD14 and showed a trend for reduction of CD4 subpopulations. However, although the decrease in CD14 seems to be a fumonisin effect, the decreases in CD4 and CD8 could be a result of the AC treatment alone. Unfortunately, because the AC only group was not analyzed, it is impossible to draw any firm conclusions as to the cause of the decrease in CD8 and CD4. However, with regard to the effects of FB alone, it seems that FB affects B cells (CD14) and T cells (CD4 and CD8) differently because there is a clear FB effect on CD14.

Table 5. Effects of fumonisin B₁ consumption with or without the addition of activated carbon (1% of the diet, as-fed basis) on urinary free sphingoid bases of weanling pigs

Item	Treatments ^a				SEM	P-value
	Control	AC	FB	AC + FB		
Sphingosine (So), nM	20.89	4.54	3.48	4.46	5.94	0.07
Sphinganine (Sa), nM	4.29 ^x	1.56 ^x	82.32 ^y	25.39 ^{xy}	16.86	0.03
Sa:So ratio	0.62 ^x	0.64 ^x	36.17 ^y	14.94 ^{xy}	7.17	0.02

^aControl = corn-soybean basal diet with < 2 ppm FB; AC = control + activated carbon at 1% of the diet, as-fed basis; FB = control + culture material (formulated to contain 30 ppm fumonisin B₁, as-fed basis); AC + FB = control + activated carbon at 1% of the diet, as-fed basis + culture material.

^{x,y,z}Within a row, means without a common superscript letter differ, $P < 0.05$.

Table 6. Effects of fumonisin B₁ consumption with or without the addition of activated carbon (1% of the diet, as-fed basis) on immunological measurements of weanling pigs^a

Monoclonal antibodies ^c	Treatment ^b			SEM	P-value
	Control	FB	AC + FB		
CD4, %	16.2 ^x	10.5 ^x	2.3 ^y	1.9	0.003
CD8, %	27.7 ^x	31.8 ^{xy}	4.5 ^y	4.5	0.019
CD14, %	14.1 ^x	6.3 ^y	2.6 ^y	1.5	0.001
CD4:CD8 ratio	0.6	0.33	0.6	0.9	0.836

^aEach value represents the mean percent of cells analyzed positive of the binding of the specific antibody for four pigs per treatment.

^bControl = corn-soybean basal diet with <2 ppm FB; FB = control + culture material (formulated to contain 30 ppm fumonisin B₁, as-fed basis); AC + FB = control + activated carbon at 1% of the diet, as-fed basis + culture material.

^cMonoclonal antibodies are mouse anti-porcine. CD4 = T helper lymphocytes; CD8 = cytotoxic T lymphocytes; CD14 = monocytes/macrophages.

^{x,y,z}Within a row, means without a common superscript letter differ, *P* < 0.05.

The mechanism responsible for the apparent fumonisin-induced decrease in CD14 may be due to FB-induced apoptosis and necrosis in lymphoid tissue. Alternatively, it could be attributed to the redistribution of the monocyte and lymphocyte cells in different organs or tissues, supported by the histological finding in this experiment and that conducted in humans with compromised immune systems by Rosenberg et al. (1998). Nonetheless, the FB-induced decrease in CD14 is most likely due to the fact that CD14 is a glycosylphosphatidylinositol (GPI)-anchored membrane protein (Pugin et al., 1998). The function of GPI protein receptors depends on lipid raft function. Lipid rafts are sphingoid-based microdomains, and their function is disrupted by inhibitors of ceramide synthase, such as FB (Wang et al., 1991). Inhibitors of ceramide synthase also have been shown to suppress T-dependent immune response (CD3, CD4, CD8, CD45), which inhibits DNA synthesis, also modifying T-lymphocyte surface antigen expression (Martinova, 1998). Recently, Oswald et al. (2003) reported an increase in bacterial colonization in the intestines of piglets exposed to FB. The authors concluded that exposure to FB predisposed the animals to infectious disease.

The dietary addition of the activated carbon had no beneficial effect when added alone or in combination to the FB-contaminated diets. Activated carbon has been used as an effective detoxification agent, and some research data suggest that it has potential as a mycotoxin-sequestering agent (Ramos and Hernandez, 1997; Galvano et al., 2001). This activated carbon product previously had been tested in vitro and was shown to have a high absorption potential for FB (Galvano et al., 1997). The ineffectiveness of the activated carbon in vivo could be due to the limited exposure of the pores to the FB or to the low specificity of the product. The low absorptive specificity of this material also could help to explain the increase in negative effects associated with its consumption. The activated carbon could absorb important nutrients, including vitamins, minerals, and essential AA, all of which play important roles

in the overall health and performance of animals. For example, AC could absorb folate, and because FB is a known inhibitor of the GPI-anchored folate transporter (Stevens and Tang, 1997), the addition of AC could exacerbate the effects of folate insufficiency.

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